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Sub-Band Deletion of 7q36.3 in a Patient With Ring Chromosome 7: Association With Holoprosencephaly

Jeffrey R. Sawyer, Janet L. Lukacs, Susan J. Hassed, Georgianne L. Arnold, Heather F. Mitchell, and Maximilian Muenke

Departments of Pathology (J.R.S.) and Pediatrics (S.J.H., G.L.A.), University of Arkansas of Medical Sciences, Cytogenetics Laboratory (J.R.S., J.L.L.), Arkansas Children's Hospital, Little Rock, Arkansas; and Division of Human Genetics and Molecular Biology (H.F.M., M.M.), Departments of Pediatrics and Genetics, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

We report on a patient with ring chromosome 7 analyzed by both high-resolution mid-prophase G-banding and fluorescence in situ hybridization (FISH) resolving a sub-band deletion of 7q36.3 associated with the clinical manifestation of holoprosencephaly (HPE). © 1996 Wiley-Liss, Inc.

KEY WORDS: ring chromosome 7, holoprosencephaly, mid-prophase, FISH

INTRODUCTION

Ring chromosome 7 is a rare cytogenetic aberration with only nine reported cases [Zackai and Breg, 1973; Nakano and Miyamoto, 1977; DeLozier et al., 1982; Barros et al., 1986; Kohyama et al., 1988; Caramia et al., 1990; Koiffmann et al., 1990; Biesecker et al., 1991; Tsukamoto et al., 1993]. The clinical findings associated with ring 7 are highly variable; however, most reported cases include growth and mental retardation, microcephaly, dermatological abnormalities including nevus flammeus, café-au-lait spots, and dark pigmented nevi. The variation in phenotypic effects in different patients suggests different causal mechanisms which could include size variations of chromosomal deletions, or abnormal chromosomal behavior such as ring instability leading to mosaicism in different tissues.

A critical region for one of the HPE genes has been mapped to 7q36 with a cosmid probe which maps less than one Mb proximal to the HPE3 gene [Gurrieri et al., 1993; Muenke, 1994]. Deletions of chromosome band 7q36 are associated with holoprosencephaly (HPE) in patients without ring 7 [Gurrieri et al., 1993]; therefore, the finding of ring 7 patients with HPE as a clinical finding is not surprising. However, HPE is not a frequent finding in patients with a ring 7 and, to our knowledge, only one previous patient with ring 7 has shown the classic manifestation of HPE [Tsukamoto et al., 1993]. This suggests that deletions large enough to include all or part of band 7q36 are not common in ring 7 patients, or that these patients did not have the central nervous system manifestations of HPE, but only a HPE microform. Unfortunately, breakpoints in ring chromosomes have been difficult to resolve with conventional cytogenetic techniques, and therefore the loss of genetic material by traditional cytogenetic techniques has not been demonstrated in any ring 7 patient. To ascertain the presence of a deletion of the ring 7 in this patient, we applied a new high-resolution G-banding technique [Sawyer, 1995] and fluorescence in situ hybridization with a cosmid probe to the putative HPE3 gene at 7q36 [Gurrieri et al., 1993]. We report the second case of a ring chromosome 7 in a patient with multiple anomalies including HPE. However, this is the first case to demonstrate a cytogenetically visible deletion by mid-prophase G-banding, confirmed by FISH analysis with the cosmid probe CEB56 which maps to the HPE3 minimal critical region in band 7q36.

Clinical Findings

The patient was born at term to a primagravid mother by cesarean section for fetal distress. Apgar scores were 3 at 1 min and 7 at 5 min. Birth weight was 2.14 kg (25th centile), length was 44 cm (<10th centile), and OFC was 30 cm (<3rd centile). The patient was transferred to a tertiary care center for evaluation of midline cleft lip and ambiguous genitalia. The physical examination showed hypotelorism, proptosis, proboscis with a single nostril, a complete midline cleft lip and palate, small ears, short neck with excess nuchal skin, hypoplastic nipples, a small penis with rugate scrotum, and normal fingers and toes with slightly hyperconvex nails (Fig. 1). Echocardiogram was normal. A computed tomography scan of the head was consistent with semilobar holoprosencephaly. The patient had intractable

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Georgianne L. Arnold is now at Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Address reprint requests to Dr. Jeffrey R. Sawyer, Cytogenetics Laboratory, Arkansas Children's Hospital, 800 Marshall Street, Little Rock, AR 72202.

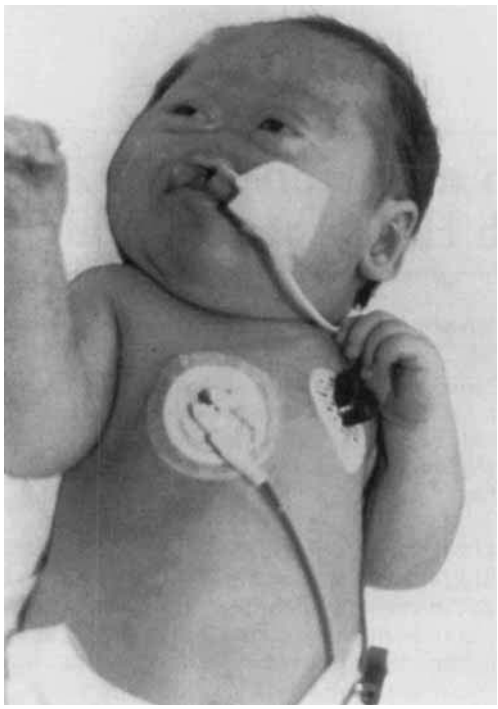

Fig. 1. The patient, demonstrating hypotelorism, with proptosis, flat proboscis, with a single nostril, and midline cleft lip.

seizures, required gastrostomy tube placement at age 2 months, and died at 5 months of pneumonia. An autopsy was not obtained.

Cytogenetic Methods and Findings

Routine chromosome analysis and FISH studies were carried out on methotrexate synchronized peripheral blood lymphocytes as previously described [Yunis et al., 1978]. Chromosome analysis of the original peripheral blood sample was performed and 100 cells were analyzed; 90 cells had a 46,XY,r(7)(p22q36) karyotype, 8 showed 45,XY,−7, 1 cell showed a 46,XY,dup r(7)(p22q36), and 1 cell showed a 47,XYr(7)(p22q36), +r(7)(p22q36) pattern. A second blood sample was obtained and mid-prophase chromosome banding studies were carried out using a combination of cell synchronization, ethidium bromide, and echinomycin [Sawyer, 1995]. A deletion of band 7q36.3 is demonstrated in examples of the mid to late-prophase ring chromosome 7 (Fig. 2).

FISH Studies

In order to define the deletion of the ring chromosome 7 on a molecular level, fluorescence in situ hybridization (FISH) studies were performed on chromosomes from a peripheral blood sample of the patient. Cosmid probe CEB56 has previously been shown to map to the holoprosencephaly (HPE) minimal critical region in 7q36 [Gurrieri et al., 1993] and more specifically to a region which maps less than one Mb proximal to HPE3, the putative HPE gene in 7q36 (Muenke et al., unpublished). This probe (CEB56) was labeled with biotin using random primer extension as recommended by the supplier (ONCOR, Inc., Gaithersburg, MD). In addition to the biotin-labeled cosmid probe, a centromere specific chromosome 7 probe (ONCOR) was used to identify the normal chromosome 7. FISH, post-hybridization washes, and fluorescence photomicroscopy were done by routine methods [Yeh et al., 1994].

Following the FISH procedure, 15 chromosome spreads were analyzed. The normal chromosome 7 identified by the chromosome 7 centromere probe had hybridization signals at the terminal end of the long arm. The ring chromosome 7 was positive only for the centromere-specific probe and negative for the cosmid probe CEB56 in all cells analyzed (Fig. 3). These FISH results confirm that the r(7) chromosome is deleted for a region in 7q36 which includes the putative HPE3 gene.

DISCUSSION

Holoprosencephaly (HPE) is a causally heterogeneous field defect resulting from failure of cleavage of the prosencephalon, often accompanied by abnormalities in midline facial development. Holoprosencephaly may occur as a sporadic malformation, as a single gene defect, as part of a multiple malformation syndrome, or as one component of a chromosomal disorder [Muenke, 1994]. Varying degrees of deficiency occur in midline facial development, ranging from hypotelorism and incomplete forebrain development to the extreme of cyclopia and grossly incomplete morphogenesis of the forebrain. This anomaly is most frequently associated with trisomy 13 but has also been found with aberrations of chromosomes 18 [Overhauser et al., 1995], duplications of 3p [Gurrieri et al., 1993], deletions of 2p21 [Schell et al., 1995], 7q [Muenke et al., 1994] and 21q22 [Muenke et al., 1995], and triploidy [Muenke et al., 1988].

The association of HPE to specific chromosome band deletions has been demonstrated at 2p21 [Sawyer et al., 1994; Schell et al., 1995] and at 7q36 [Gurrieri et al., 1993]. However, HPE in a patient with ring 7 has been reported only once previously [Tsukamoto et al., 1993]. The wide variation of phenotypic findings in patients with ring chromosome 7 is probably due to several factors. Since most of these patients do not have HPE, it is likely that there is no deletion of the HPE3 gene at 7q36, and that the deletion event must be telomeric in most patients. Alternatively, the deletion may involve a very small segment, or, in patients with nearly normal phenotypes, there may be no loss of chromosome material, suggesting telomeric fusion.

The mechanics of ring chromosome formation may provide a partial explanation for the phenotypic variations. There are two mechanisms that result in ring chromosome formation. The most common mechanism is breakage in both arms of the chromosome with loss of distal segments and subsequent fusion of the ends. This results in the loss of chromosomal material and generally produces multiple phenotypic effects. The second type of ring chromosome is caused by telomere-to-telomere fusion, which may result in little or no loss of chromatin and may have significant consequences only if the ring chromosome is lost, resulting in mono-

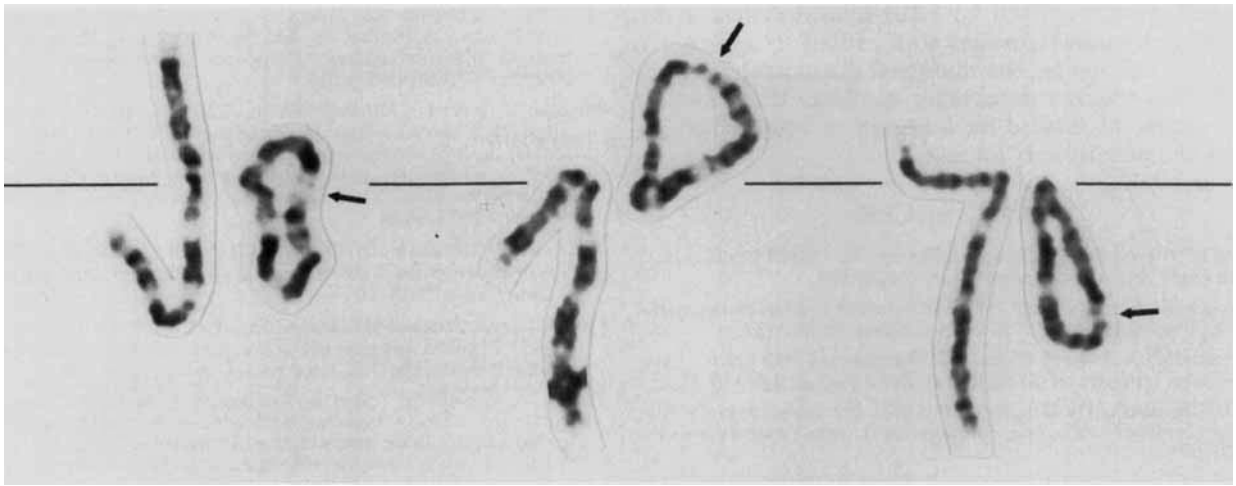

Fig. 2. Three selected pairs of mid-prophase chromosomes 7. Arrows indicate point of deletion in the ring. G-positive sub-bands 7q36.2 and 7p22.3 are visible delimiting the deletion to 7q36.3 at the chromosome banding level.

somy. This form of ring formation is the type thought to be responsible for the "ring chromosome syndrome" proposed by Côté et al. [1981] and Kosztolányi [1987]. The "ring syndrome" hypothesis postulates that the phenotype is independent of the chromosome involved and the clinical findings are not a result of the loss of genetic material, but of decreased viability likely caused by aneuploid cells which occur de novo rather than in a clonal manner [Kosztolányi, 1987; Kosztolány et al., 1991]. The instability of ring chromosomes is thought to be caused by sister chromatid exchange within the ring, which apparently results in unstable variants, including intermediate dicentric chromosomes. Recent studies using telomeric and subtelomeric sequence probes support this hypothesis providing evidence that the so-called "ring syndrome" results from telomere fusion, and subsequent ring chromosome instability [Pezzolo, 1993]. If cases of ring 7 occur by telomeric fusion, then we speculate chromosome instability is the likely explanation for the phenotypic variability in these cases.

In the present case, a deletion of part of band 7q36 is demonstrated both by high-resolution mid-prophase G-banding and FISH analysis. The abnormal behavior of the ring chromosome has apparently produced duplications and double rings that were subsequently lost, leading to the 5–7% of the cells with a 45,XX,−7 karyotype. The deletion of HPE3 and genes distal to that locus may have contributed to produce the phenotypic effects seen in our patient. The resolution of breakpoints of the ring at the mid-late prophase sub-band level demonstrates the deletion at band 7q36.3 on the long arm (Fig. 2). The FISH analysis with a cosmid probe to HPE3 confirmed this deletion in 7q36 (Fig. 3). A detailed physical map of 7q36 has been made by Gurrieri et al. [1993], which indicates this area contains a gene or genes critical for the development of forebrain midline structures. The lack of HPE as an anomaly in most ring 7 patients suggests that most patients have more distal deletions that do not include 7q36.3 or have telomeric fusions which are more consistent with the "ring syndrome" hypothesis.

NOTE ADDED IN PROOF

After submission of this manuscript we have performed Southern blot analysis of *Hinf* I-digested DNA from the patient and his parents. The highly polymorphic probe pλg3 (locus: D722) from the HPE3 minimal

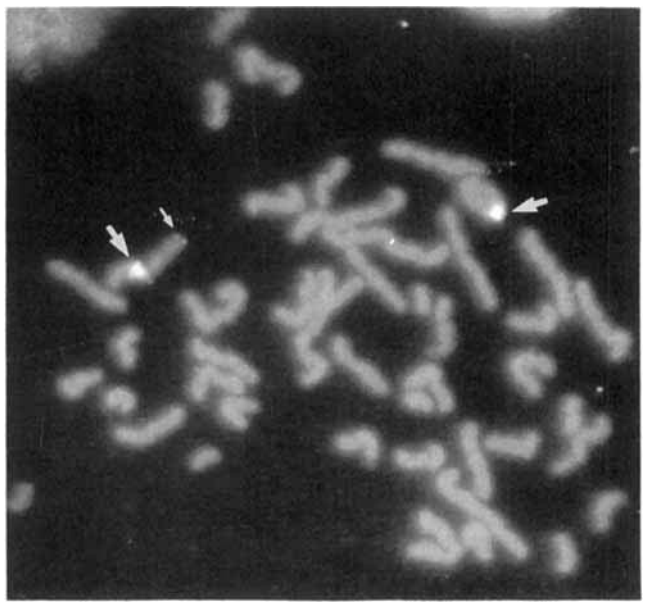

Fig. 3. Fluorescence in situ hybridization (FISH) analysis of cosmid probe CEB56 to metaphase chromosomes of the patient with a ring r(7) chromosome. The normal chromosome 7 and the r(7) show a hybridization signal from the centromere-specific probe (large arrows). In addition, the normal chromosome 7 is positive for CEB56 (small arrow). In contrast, the r(7) chromosome is deleted for CEB56, suggesting that the putative holoprosencephaly gene, HPE3, in 7q36 is also deleted in this patient.

critical region was used for filter hybridization as described previously [Gurrieri et al., 1993]. The patient's DNA was deleted for the maternal allele at the D7S22 locus. This result furthermore confirms that the r(7) chromosome is deleted for a region in 7q36 which includes the putative HPE3 gene.

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